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EDITORIAL COMMENT

Visualizing the RAGE

Molecular Imaging After MI Provides Insight Into a Complex Receptor*

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Changes in left ventricular (LV) myocardial structure (remodeling) often occurs after a myocardial infarction (MI) and contributes to the progression to heart failure. Although it is clear there is no single pathway or event that contributes to adverse LV remodeling after MI, the inflammatory cascade that entails the release of biologically active molecules (cytokines) likely plays a central role in this process. Specifically, while inflammation is critical for the appropriate wound healing response, the

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biological mediators attendant to the inflammatory response also likely contribute to adverse remodeling through a number of intracellular and extracellular proteolytic pathways. Thus, the early post-MI period can yield a feed-forward process of cellular and extracellular events that initiate and promulgate the adverse remodeling process. Peripheral blood sampling can provide invaluable information on the initial degree of myocardial injury and the magnitude of the inflammatory response. However, these surrogate chemical measurements do not provide spatial specificity, and therefore do not provide critical information on the anatomic location of the region or regions undergoing or at risk for undergoing adverse LV remodeling. In this issue of *iJACC*, Tekabe et al. (1) have addressed this research goal through a feasibility study whereby a specific receptor, the receptor for advanced glycation end products (RAGE), that is likely evoked early in the post-MI inflammatory-signaling cascade was imaged in a murine MI model.

RAGE signaling and relevance to MI. Requisite events in the LV remodeling process include changes in protein stability and turnover, which in turn will alter cellular and extracellular structure-function relationships. A clear example of changes in protein stability and turnover is that of post-translational modification, which occurs with the formation of advanced glycation end products (AGEs). One consequence of changes in protein turnover and cross-linking occurring with AGEs is fibrillar collagen, whereby increased collagen accumulation can occur, causing myocardial fibrosis and abnormalities in LV diastolic function (2). Although post-translational modification of proteins was initially considered to be the primary consequence of AGE formation, AGEs serve as ligands for a transmembrane receptor, namely, RAGE. As RAGE is a member of the superfamily of immunoglobulin receptors, it therefore is not surprising that there are multiple ligands for RAGE. In particular, it has been documented that specific components of the inflammatory cascade, such as S100B, serve as ligands for RAGE (3). This may be of particular relevance with respect to ischemia-reperfusion as S100B can be released as a consequence of necrotic cell death, apoptosis, and autophagy—all likely occurring in this pathological context. Thus, RAGE can be activated after MI without the absolute presence of AGEs. Intracellular transduction of RAGE appears to be through the classical mitogen-activated protein kinases and extracellular signal-regulated kinases, resulting in the formation of transcription factors such as nuclear factor kappa B. This receptor transduction pathway for RAGE would, therefore, likely cause the elaboration of proinflammatory cytokines. Thus, the up-regulation

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of RAGE would in turn cause a number of biological cascades that would contribute to the adverse LV remodeling process with MI. Indeed, in transgenic mice with gene deletion for RAGE, the initial LV remodeling and dysfunction that invariably occurs in the murine MI model was ameliorated (4). Furthermore, the use of a ligand decoy and a soluble RAGE ligand could also attenuate early post-MI remodeling in this mouse model (4). The study by Tekabe et al. (1) represents a fundamental leap forward with respect to RAGE biology and post-MI remodeling by developing a method to visualize RAGE expression within the intact murine system through the use of a radiolabeled RAGE antibody. Through this approach, these investigators provide the first demonstration in vivo that an up-regulation of RAGE occurs early post-MI and is localized to the region of the left ventricle at most risk for adverse remodeling.

Visualizing RAGE and MI. In the study by Tekabe et al. (1), dual isotope imaging using single-photon emission computed tomography/computed tomography (SPECT/CT) was performed in a rat model of ischemia-reperfusion and subsequent MI. For the purposes of visualizing RAGE, a fragmented antibody targeted against the extracellular V-domain was conjugated with ^{99m}Tc (^{99m}Tc-antiRAGE) and injected at ~20 h or 48 h post-MI induction. At 5 h after this initial injection, thallium-201 was injected and dual SPECT/CT performed. The investigators reported more than a fourfold increase in ^{99m}Tc-antiRAGE uptake within the MI region at ~20 h post-MI, which, while remaining detectable, was significantly diminished by 48 h post-MI. Ex vivo well counting confirmed these in vivo imaging results, and immunohistochemistry demonstrated localization to the site of ongoing myocardial injury and remodeling. The investigators reported that the RAGE localization was particularly prevalent where myocyte apoptosis may be occurring within the MI region.

This study is significant from both a basic and a translational research perspective. From the basic science perspective, this study clearly demonstrated that an induction of RAGE occurs after myocardial injury and that the presence of AGEs is not a requisite for this receptor up-regulation. The localization of RAGE to the site of myocardial injury is consistent with the induction of the inflammatory cascade that occurs early post-MI, but more importantly, may have been co-localized to cardiac myocytes undergoing apoptosis. These observations would, therefore, support the postulate that the release of intracellular S100B by cells undergoing apoptosis would provide a ligand for the RAGE receptor. From a translational research perspective, these studies demonstrate the feasibility of quantifying biological events in an intact physiological system that holds relevance to post-MI remodeling.

Other studies have similarly identified the feasibility of imaging matrix metalloproteinase activity and angiogenesis in rodent models of MI (5–7). As with any feasibility study of this type, the study by Tekabe et al. (1) identifies a number of issues that will warrant investigation in the future. First, what are the critical cell types that express RAGE in the post-MI period that, in turn, directly contribute to the post-MI remodeling process? The current study colocalized RAGE to cardiomyocytes presumably undergoing apoptosis, but a major cell type that expresses RAGE is the macrophage. In fact, the same investigators reported in another murine model of cardiovascular disease that the ^{99m}Tc-antiRAGE signal was primarily localized to the macrophage (6). The macrophage, through direct and indirect effects, plays a significant role in the post-MI remodeling process; thus, whether up-regulation of RAGE in these cells is a relevant signaling pathway remains to be established. A limitation of the present study was the slow clearance of ^{99m}Tc-antiRAGE from the blood pool, which restricted the temporal resolution by which these studies could be performed, and further required a significant delay for the parallel perfusion imaging studies. Nevertheless, this study provides convincing imaging that an early up-regulation of RAGE occurs in a small animal model of MI. Future studies that induce hyperglycemia and the formation of AGEs in this rodent MI model would be a provocative direction to take with these RAGE imaging studies.

The study by Tekabe et al. (1) continues to provide a solid foundation and proof of concept that in vivo imaging of biological signaling cascades and pathways that are induced after MI is feasible. These basic imaging studies set the foundation by which to determine the relationship between in vivo quantification of biological signaling events to the progression and magnitude of adverse LV remodeling. The ability to perform molecular imaging coupled with measurements of LV geometry and function most certainly hold relevance to developing predictive models for prognosis and eventual treatment efficacy in the post-MI period. Of course, extrapolating a rodent study to clinical applications must be done with great caution, and up-titration of

these studies to more clinically relevant large-animal model imaging will be essential. However, our laboratory has demonstrated that translation of murine imaging to clinically relevant post-MI models can be successfully performed (7). Thus, it would seem likely that clinical feasibility studies that perform imaging of cellular and molecular events, such as those described in the study presented in this issue of *iJACC* (1), as well as by others (5–7), are in the not too distant future.

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Key Words: advanced glycation end products ■ LV remodeling ■ myocardial infarction ■ RAGE ■ SPECT/CT.